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Award Number: DAMD17-01-1-0013

TITLE: Enhanced Peptide Radiotherapy of Prostate Cancer Using Targeted Adenoviral Vectors

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REPORT DATE: June 2004

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2004	Annual (1 Jun 03 - 31 May 04)

4. TITLE AND SUBTITLE
Enhanced Peptide Radiotherapy of Prostate Cancer Using Targeted Adenoviral Vectors

5. FUNDING NUMBERS
DAMD17-01-1-0013

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

An adenovirus encoding the genes for human somatostatin receptor subtype 2 has been constructed and evaluated in human prostate cancer cells with regard to binding of ^{64}Cu-octreotide. In vitro experiments were performed with DU-145 and PC-3 human prostate cancer cells. Expression levels of SSTR2 were determined using a ^{64}Cu-octreotide saturation binding assay on cell membrane preparations. In vivo experiments were conducted in scid mice bearing subcutaneous DU-145 or PC-3 cells. AdSSTR2 was injected intratumorally followed 48 h later by an i.v. injection of ^{64}Cu-octreotide. The mice were sacrificed 1 h after peptide injection for biodistribution analysis. The expression of SSTR2 on DU-145 cells was 9485 fmol/mg after infection at 100 MOI compared to 3540 fmol/mg on PC-3 cells. In vivo biodistribution studies showed similar uptake of ^{64}Cu-octreotide in both DU-145 and PC-3 tumors after infection with AdSSTR2 (2.5 and 2.7% ID/g, respectively). This uptake was greater than that observed in tumors injected with control adenovirus (1.4 - 1.6% ID/g).

14. SUBJECT TERMS
Prostate cancer, adenovirus, gene transfer, somatostatin radiolabeled peptides

15. NUMBER OF PAGES
17

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

It is estimated that approximately 37,000 U.S. men died in 1999 from prostate cancer. It is clear that novel treatments for prostate cancer are necessary. Radiolabeled monoclonal antibodies have been used to treat hormone-refractory prostate cancer with limited success. Reasons for these limitations include, bone marrow toxicity from the long serum half-life of the radiolabeled antibody, heterogeneous tumor distribution of the large molecular weight antibody, and low tumor antigen/receptor expression. A strategy to overcome these limitations is to combine peptide radiotherapy with gene therapy. Radiolabeled peptides can overcome problems associated with bone marrow toxicity and tumor penetration due to their small molecular weight, while gene therapy can be used to increase the tumor antigen/receptor expression. Previous studies have shown that an adenovirus encoding for the somatostatin receptor subtype 2 (AdSSTR2) can be used to increase tumor localization of radiolabeled octreotide analogues. **Objective/Hypothesis.** The objective of this proposal is to determine if induction of SSTR2 with AdSSTR2 on human prostate cancer xenografts in mice has a therapeutic effect after targeting with the octreotide analogue, ^{64}Cu-octreotide. **Specific Aims.** SPECIFIC AIM #1. Evaluate the expression of SSTr2 on human prostate cancer cells *in vitro* after infection with AdSSTR2 using radiolabeled octreotide binding and internalization assays. SPECIFIC AIM #2. Evaluate the distribution of radiolabeled octreotide after i.v. injection by non-invasive PET imaging and by gamma counter analysis in nude mice bearing s.c. human prostate cancer xenografts injected with AdSSTR2. SPECIFIC AIM #3. Perform therapy studies in a mouse model of human prostate cancer utilizing the best vector as determined in Specific Aims 1 and 2 and ^{64}Cu-octreotide. **Study Design.** The first aim of the study will evaluate SSTR2 expression on PC-3 and DU-145 human prostate cancer cells *in vitro* after infection with AdSSTR2. These assays will be conducted using ^{64}Cu-octreotide in Scatchard and internalization experiments. The Scatchard analysis will determine the level of SSTR2 expression on the cells and the internalization of SSTR2 is important for the subsequent localization and therapy studies. The PC-3 and DU-145 cells will then be implanted s.c. in athymic nude mice and SSTR2 expression will be determined by ^{64}Cu-octreotide tumor localization following injection of AdSSTR2. These studies will be conducted using PET imaging, tissue counting in a gamma counter and immunohistochemistry. Therapy will be conducted in mice bearing subcutaneous PC-3 and DU-145 tumors following injection of AdSSTR2 and i.v. injections of ^{64}Cu-octreotide. **Relevance.**

These studies are directly relevant to improving the treatment of hormone-refractory prostate cancer. Novel therapies are needed for the treatment of this disease and this proposal introduces a new paradigm for its treatment by combining targeted radiolabeled peptide therapy with gene therapy. In addition, this strategy can be used to detect prostate cancer using external PET imaging.

Body

Statement of Work

SPECIFIC AIM #1. Evaluate the expression of SSTr2 on human prostate cancer cells *in vitro* after infection with AdSSTr2 using radiolabeled octreotide binding and internalization assays.

Task1: Months 1-4: Radiolabel octreotide with ^{64}Cu and use the ^{64}Cu-octreotide to determine the level of SSTr2 expression in PC-3 and DU-145 human prostate cancer cells after infection with various amounts of AdSSTr2. This will be done by Scatchard analysis. In addition, internalization of SSTr2 and ^{64}Cu-octreotide will be evaluated.

SPECIFIC AIM #2. Evaluate the distribution of radiolabeled octreotide after i.v. injection by non-invasive PET imaging and by gamma counter analysis in nude mice bearing s.c. human prostate cancer xenografts injected and with AdSSTr2.

Task 1: Months 3-12: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected intratumorally with AdSSTr2 3-5 weeks later. ^{64}Cu-octreotide will then be administered i.v. and the mice imaged using micro PET to determine SSTr2 expression and ^{64}Cu-octroetide distribution.

Task 2: Months 3-12: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected intratumorally with AdSSTr2 3-5 weeks later. ^{64}Cu-octreotide will then be administered i.v. and the mice will be sacrificed to determine SSTr2 expression by immunohistochemistry and counting tissues in a gamma counter. These studies will be complementary to those discussed in Task 1.

Task 3: Months 6-15: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected **i.v.** with AdSSTr2 3-5 weeks later. ^{64}Cu-octreotide will then be administered i.v. and the mice will be sacrificed to determine SSTr2 expression by immunohistochemistry and counting tissues in a gamma counter. These studies will be an initial step towards evaluating this system in the context of hormone-refractory disease. Administration of the vectors i.v. will not be used in therapy studies unless the tumor expression of SSTr2 is at

least two-fold greater than expression in the liver.

SPECIFIC AIM #3. Perform therapy studies in a mouse model of human prostate cancer utilizing the best vector as determined in Specific Aims 1 and 2 and ^{64}Cu-octreotide.

Task 1: Months 13-24: Athymic nude mice will be implanted s.c. with PC-3 and DU-145 human prostate cancer cells and injected intratumorally with AdSSTr2 3-5 weeks later. Various therapeutic doses of ^{64}Cu-octreotide will be administered i.v. 2 and 4 days after adenovirus. Tumors will be measured every 3 days to determine if there is any response to treatment. Controls will include an irrelevant adenovirus injection, unlabeled octreotide injections, and no treatment. Toxicity will also be monitored throughout the studies.

Since the previous annual report, the PI has evaluated AdSSTR2 in human prostate cancer cells for binding of ^{64}Cu-octreotide. In addition, human tumor xenografts were established in scid mice and the biodistribution of ^{64}Cu-octreotide was evaluated after intratumoral injection of AdSSTR2. These studies are directly related to completing Specific Aims 1 and 2. In particular, Specific Aim #1 is now complete except for the internalization studies. For Specific Aim #2, Task 2 has been completed and it is anticipated that Tasks 1 and 3 will be completed in the next few months.

Methods

Determination of SSTR2 expression. The induction of SSTR2 in human prostate cancer cell lines was evaluated using a radiolabeled peptide binding assay to cell membrane preparations of cells that had been infected with AdSSTR2. The cells were seeded such that they were ~80% confluent at the time they were infected with AdSSTR2 and then harvested for membrane preparations 2 days after adenoviral infection. The AdSSTR2 (100 pfu per cell (MOI) for both DU-145 and PC-3 cells) was added to cells in Optimem® (Gibco-BRL, Grand Island, NY) and incubated at 37°C in 5% CO_2 for 2 h. The cells were then supplemented with complete media and incubated an additional 48 h at 37°C. Cell membranes were then prepared from the infected and uninfected cells using a protocol similar to that previously described. Briefly, the cells were washed with phosphate buffered saline, scraped from the flask, and centrifuged at 90 x g for 5 min at 4°C. The pellet was resuspended in cold lysis buffer (10 mM

Tris-HCl, 2 mM EDTA, 2 mM $MgCl_2$, pH 7.2) containing 0.5 mM phenylmethylsulfonyl fluoride and incubated on ice for 15 min. The mixture was vortexed, centrifuged at 600 x g for 15 min at 4°C, and the supernatant removed and stored on ice. An additional lysis step was performed on the pellet and the two supernatants were combined. The supernatant was centrifuged at 28,000 x g for 30 min at 4°C, the resulting supernatant discarded, and the pellet resuspended in 250 mM sucrose, 20 mM glycylglycine, and 1 mM $MgCl_2$. A BioRad (Hercules, CA) protein assay was performed to determine the protein concentration and the samples aliquoted and stored at -80°C.

For the binding assays, the membrane preparations were thawed and diluted in buffer (10 mM HEPES, 5 mM $MgCl_2$, 1 mM EDTA and 0.1% bovine serum albumin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 0.5 μg/ml aprotinin, and 200 μg/ml bacitracin, pH 7.4) to 20 μg per sample. Individual samples were added to Multiscreen Durapore filtration plates (type FB, 1.0 μm borosilicate glass fiber over 1.2 μm Durapore membrane; Millipore, Bedford, MA) and washed with buffer (10 mM HEPES, 5 mM $MgCl_2$, 1 mM EDTA and 0.1% bovine serum albumin, ph 7.4). One hundred μl ^{64}Cu-octreotide was added to each well such that the final concentration ranged between 0.1 and 500 nM. The membrane preparations were incubated for 1 h at room temperature in the presence or absence of excess competitor. The samples were washed twice with ice-cold buffer, the filters allowed to dry, and the individual wells punched out and counted in a gamma counter. The receptor density (B_{max}) was calculated using the GraphPad Prism software (San Diego, CA).

In vivo biodistribution studies. Biodistribution studies were performed in scid nude mice bearing s.c. PC-3 or DU-145 tumors. The mice were injected s.c. with 1 x 10^7 PC-3 or DU-145 cells (1:1 mixture with matrigel) followed by intratumoral injection with 3 x 10^8 pfu of AdSSTR2 after the tumors were established. Two days after adenoviral injection, the mice were injected i.v. with ^{64}Cu-octreotide (10 μCi) and sacrificed 1 h later. The tissues were then harvested and counted in a gamma counter to determine the biodistribution as the % injected dose per gram of tissue (% ID/g).

Results

The expression of SSTR2 after infection with AdSSTR2 at 100 MOI in DU-145 and PC-3 cell membrane preparations is 9485 ± 1689 fmol/mg and 3540 ± 1090 fmol/mg respectively.

Representative saturation binding curves for DU-145 and PC-3 cell membrane preparations are shown in **Figures 1** and **2**. The DU-145 cells had a significantly higher expression of SSTR2 than the PC-3 cells ($p < 0.003$). Both cell lines demonstrated a high binding affinity for ^{64}Cu-octreotide with Kd values of 21.2 ± 12.8 nM and 33.1 ± 25.1 nM for DU-145 and PC-3 respectively.

The biodistribution studies demonstrate that SSTR2 is expressed in both DU-145 and PC-3 tumors after intratumoral injection of AdSSTR2. **Figures 3** and **4** show uptake of ^{64}Cu-octreotide in DU-145 and PC-3 tumors that were injected with AdSSTR2 that was greater than uptake in tumors injected with a control adenovirus. DU-145 tumors injected with AdSSTR2 showed 2.5 ± 1.6% ID/g compared to 2.7 ± 0.7% ID/g in PC-3 cells. This differs from the in vitro cell study that showed significantly greater expression of SSTR2 in DU-145 cells. DU-145 and PC-3 cells injected with a control adenovirus showed 1.4 ± 0.1% ID/g and 1.6 ± 0.2% ID/g respectively.

Key Research Accomplishments

- Expression of SSTR2 was confirmed in both DU-145 and PC-3 human prostate cancer cells after infection with AdSSTR2 at 100 MOI using ^{64}Cu-octreotide.
- These studies showed that DU-145 expressed higher levels of SSTR2 than PC-3 cells and that ^{64}Cu-octreotide bound with high affinity.
- Expression of SSTR2 in DU-145 and PC-3 tumor xenografts after intratumoral injection of AdSSTR2 could be detected by binding of ^{64}Cu-octreotide in a biodistribution study.
- The uptake of 64Cu-octreotide was similar for DU-145 and PC-3 tumors injected with AdSSTR2 that differed from the in vitro results that showed greater uptake in the DU-145 cells.

Reportable Outcomes

None

Conclusions

These studies demonstrate that AdSSTR2 can infect both DU-145 and PC-3 human prostate cancer cells and result in expression of SSTR2. This was demonstrated through binding of ^{64}Cu-octreotide. In addition, infection of DU-145 and PC-3 tumors with AdSSTR2 results in expression of SSTR2 as evidenced by ^{64}Cu-octreotide binding in biodistribution studies. These studies have resulted in the completion of most of Specific Aim #1 and Task 2 of Specific Aim #2. We anticipate that we will be able to complete Specific Aim #1 and Tasks 1 and 3 of Specific Aim #2 in a timely manner in accordance with the Statement of Work.

Appendices

Figure Legend

Figures

Figure Legend

Figure 1. Representative ^{64}Cu-octreotide saturation binding curve in DU-145 cells. DU-145 cell membrane preparations were incubated with increasing concentrations of ^{64}Cu-octreotide in the presence or absence of excess competitor to determine nonspecific binding. Data represent the specific binding in cpm for the mean of triplicate measurements ± standard deviation.

Figure 2. Representative ^{64}Cu-octreotide saturation binding curve in PC-3 cells. PC-3 cell membrane preparations were incubated with increasing concentrations of ^{64}Cu-octreotide in the presence or absence of excess competitor to determine nonspecific binding. Data represent the specific binding in cpm for the mean of triplicate measurements ± standard deviation.

Figure 3. Biodistribution of ^{64}Cu-octreotide in scid mice bearing DU-145 tumors. Tumors were injected intratumorally with 3 x10^8 pfu of AdSSTR2 or a control adenovirus followed by i.v. injection of ^{64}Cu-octreotide 2 days later. The mice were sacrificed 1 h after injection of ^{64}Cu-octreotide and tissues harvested, weighed and counted in a gamma counter. Data represent the mean ± standard deviation (n = 4-6 animals).

Figure 4. Biodistribution of ^{64}Cu-octreotide in scid mice bearing PC-3 tumors. Tumors were injected intratumorally with 3 x10^8 pfu of AdSSTR2 or a control adenovirus followed by i.v. injection of ^{64}Cu-octreotide 2 days later. The mice were sacrificed 1 h after injection of ^{64}Cu-octreotide and tissues harvested, weighed and counted in a gamma counter. Data represent the mean ± standard deviation (n = 4-6 animals).

Figure 1

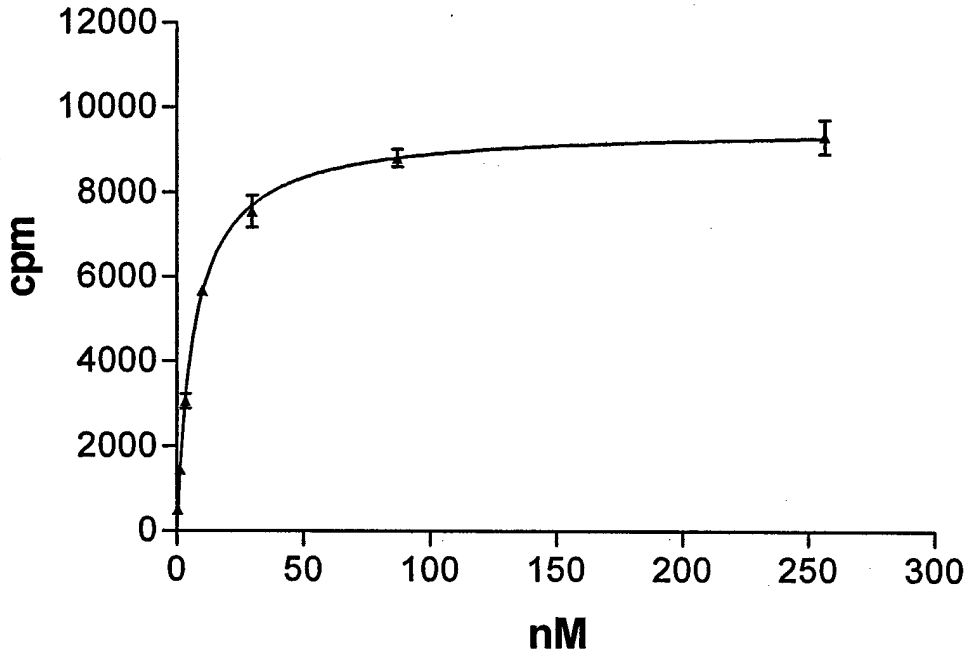
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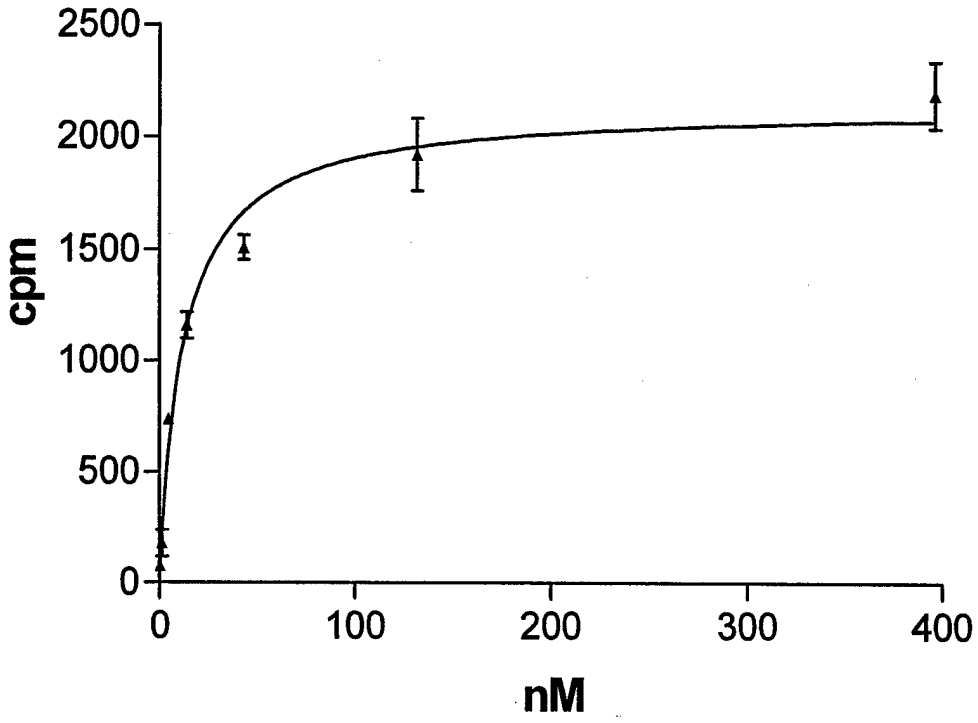
Figure 2



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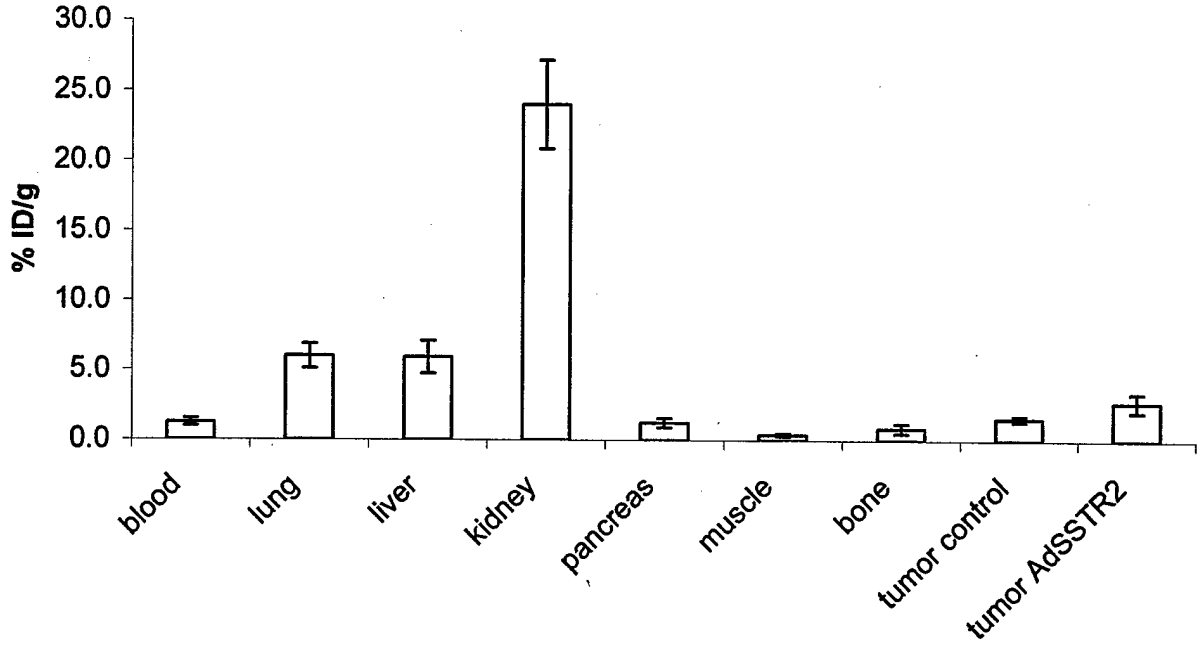
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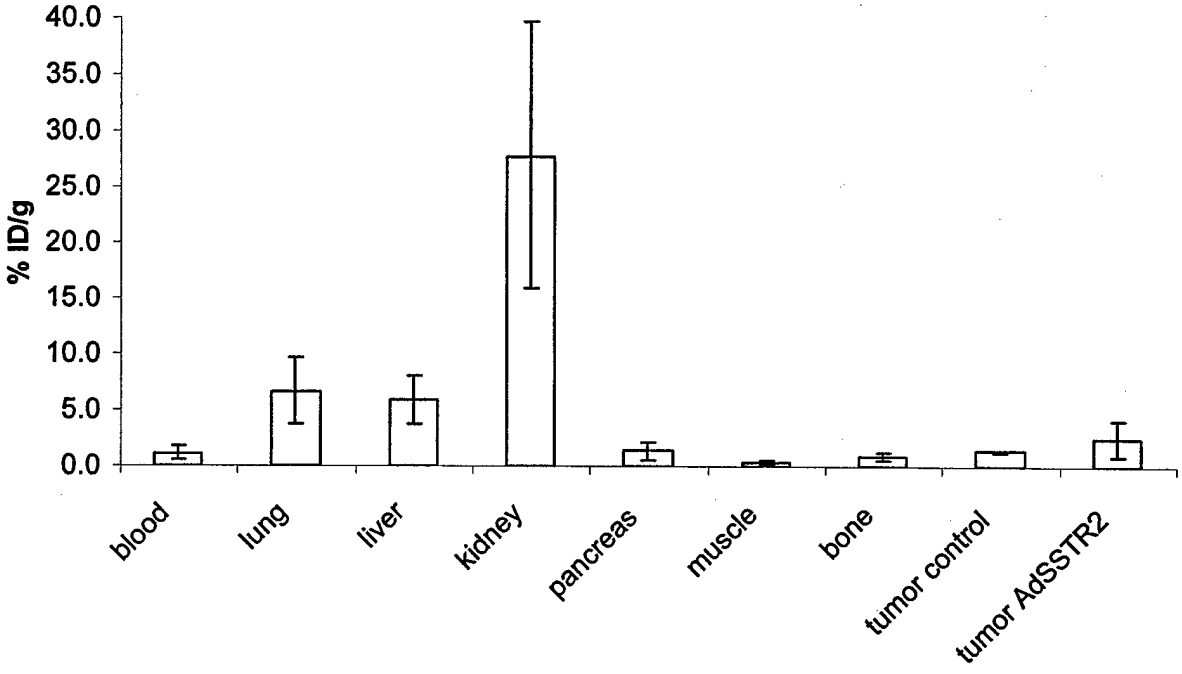
Figure 4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